

FIG. 1A

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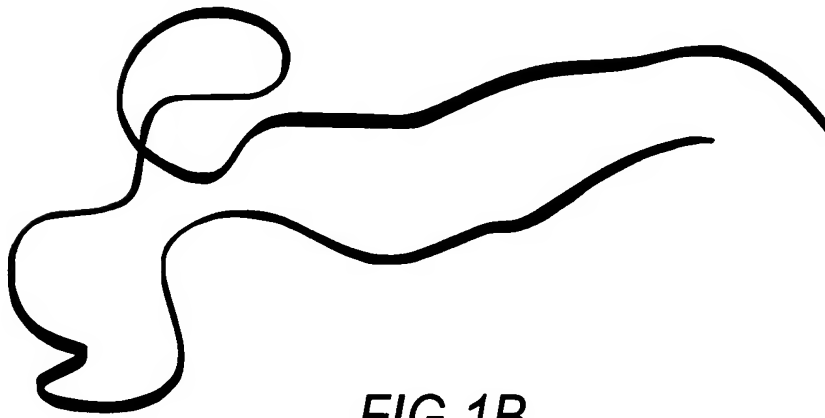


FIG.1B

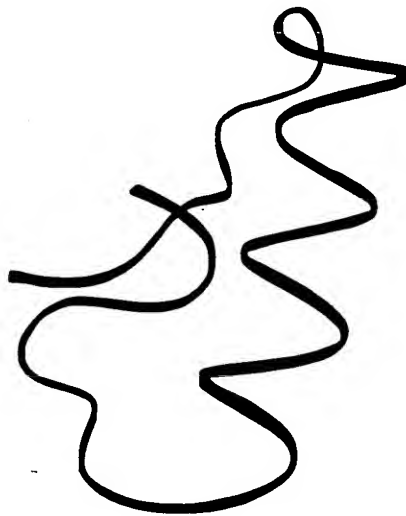


FIG.1C

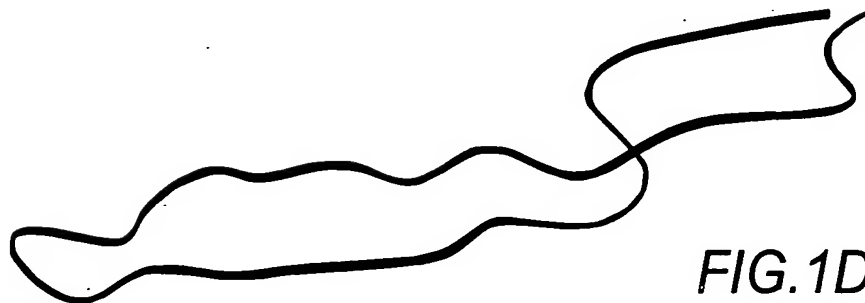
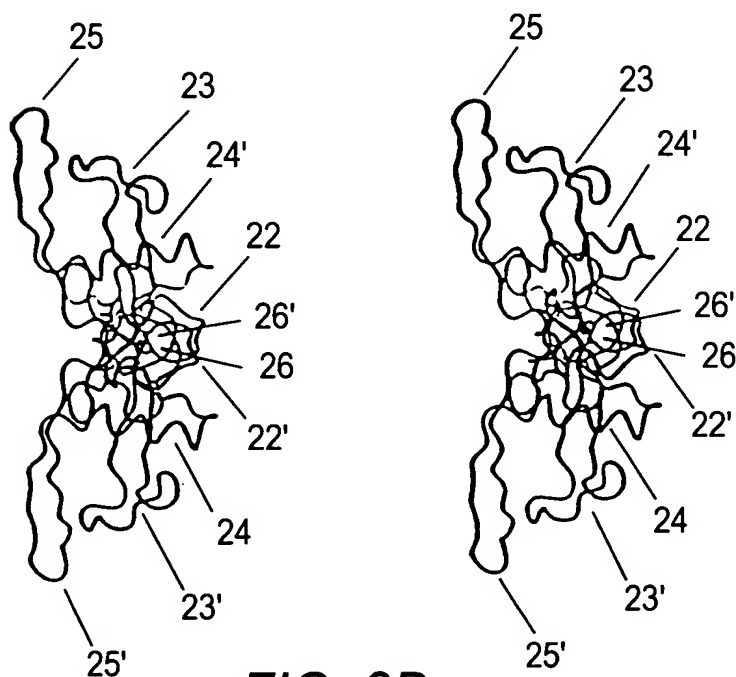
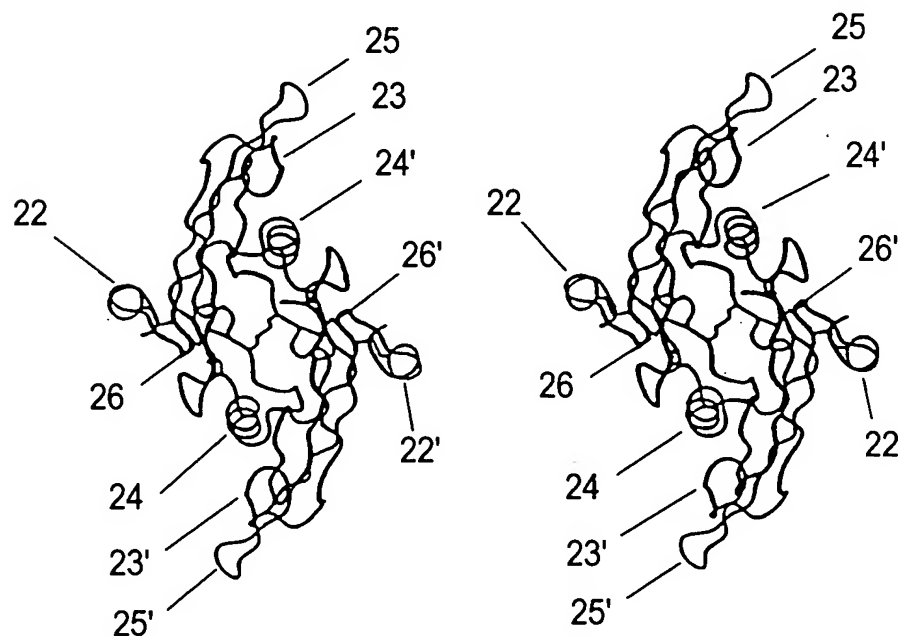


FIG.1D

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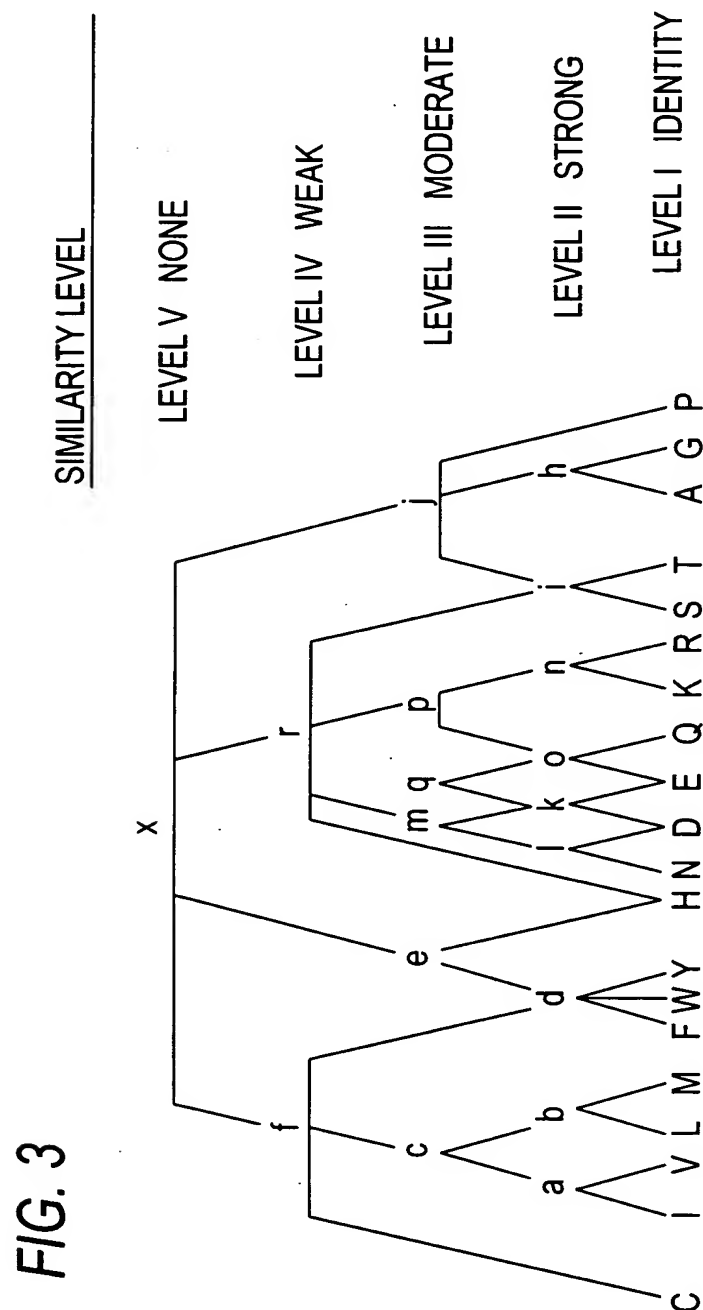
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Attorney: Karen Mangasarian (Reg. No. 43,772)

For: MODIFIED TGF- β SUPERFAMILY PROTEINS

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FIG. 4

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OP-1	CCA - - PTQLNAI SVLYFDDS- SNVI LKKYRNMVVRA	CGCH
BMP-5	CCA - - PTKLNAI SVLYFDDS- SNVI LKKYRNMVVRS	CGCH
BMP-6	CCA - - PTKLNAI SVLYFDDN- SNVI LKKYRNMVVRA	CGCH
OP-2	CCA - - PTKLSATSVLYYDSS- NNVI LRKHRNMVVKAC	CGCH
OP-3	CCV - - PTELSAI SLLYYDRN- NNVI LRRERNMVVQAC	CGCH
60A	CCA - - PTRLGALPVL YHLND- ENVNLKKYRNMI VKS	CGCH
Vg-1	CCV - - PTKMSPI SMLFYDNN- DNVVLRHYENMAVDE	CGCR
UNIVIN	CCA - - PTKLSGI SMLYFDNN- ENVVLRQYEDMVVEAC	CGCR
BMP-2	CCV - - PTELSAI SMLYLDEN- EKVVLKNYQDMVVEG	CGCR
BMP-4	CCV - - PTELSAI SMLYLDEY- DKVVLKNYQEMVVEG	CGCR
GDF-5	CCV - - PTRLSPI SILFIDSA- NNVVYKQYEDMVVES	CGCR
GDF-6	CCV - - PTKLTPI SILYIDAG- NNVVYKQYEDMVVES	CGCR
GDF-7	CCV - - PARLSPI SILYIDAA- NNVVYKQYEDMVVEAC	CGCR
CDMP-2	CCV - - PTKLTPI SILYIDAG- NNVVYNEYEEMVVES	CGCR
dpp	CCV - - PTQLDSVAMLYLNDQ- STVVLKNYQEMTVVG	CGCR
BMP-9	CCV - - PTKLSPI SVLYKDDMGVPTLKYHYEGMSVAE	CGCR
DORSALIN	CCV - - PTKLDAI SILYKDDAGVPTLIYNYEGMKVAE	CGCR
BMP-10	CCV - - PTKLEPI SILYLDKG- VVTYKFKYEGMAVSE	CGCR
GDF-3	VCCV - - PTKLSPI SMLYQDSD- KNVI LRHYEDMVVDE	CGCG
GDF-1	CCV - - PERLSPI SVLFFDNE- DNVVLRHYEDMVVDE	CGCR
SCREW	CCV - - PTVLGAI TILRYLNE- DIIDLTKYQKAVAKE	CGCH
BMP-3	CCV - - PEKMSSL SILFFDEN- KNVVLKVYPNMTVES	CA CR
NODAL	CCA - - PVKTKPLSMLYVDN- - GRVLL EHHKDMI VEE	CGCL
TGF- α 2	CCV - - SQDLEPLTILYYIG- - KTPKIEQLSNMI VKS	CKCS
TGF- α 3	CCV - - PQDLEPLTILYYVG- - RTPKVEQLSNMVVK	CKCS
TGF- α 4	CCV - - PQTLDPLPIIYYVG- - RNVRVEQLSNMVVR	ACKCS
TGF- α 1	CCV - - PQALEPLPIVYYVG- - RKPKVEQLSNMI VRS	CKCS
TGF- α 5	CCV - - PDVLEPLPIIYYVG- - RTAKVEQLSNMVVR	SCNCS
GDF-9	SICV - - PGKYSPLSVLTI EPD- GSIAYKEYEDMI ATR	CTCR
Inhibin α	CCAALPGTMRPLHVRTTSDGGYSFKYETVPNLLTQH	CACI
Inhibin β A	CCV - - PTKLRPMSMLYYDDG- QNI I KKDI QNMI VEE	CGCS
Inhibin β B	CCI - - PTKLSTMSMLYFDDE- YNI VKRDVPNMI VEE	CGCA
Inhibin β C	CCV - - PTARRPLSLLYYDRD- SNI VKTDI PDMVVEA	CGCS
MIS	CCV - - PTATAGKLLI SLSE- - ERI SAHVPNMVATE	CGCR
GDNF	CCR - - PIAFDDD- - LSFLD- - DNLVYHI LRKHS	AKRCGI
BMP-11	CCT - - PTKMSPINMLYFNDK- QQI I YGKI PGMVVD	RCGCS
GDF-9	SICV - - PGKYSPLSVLTI EPD- GSIAYKEYEDMI ATR	CTCR

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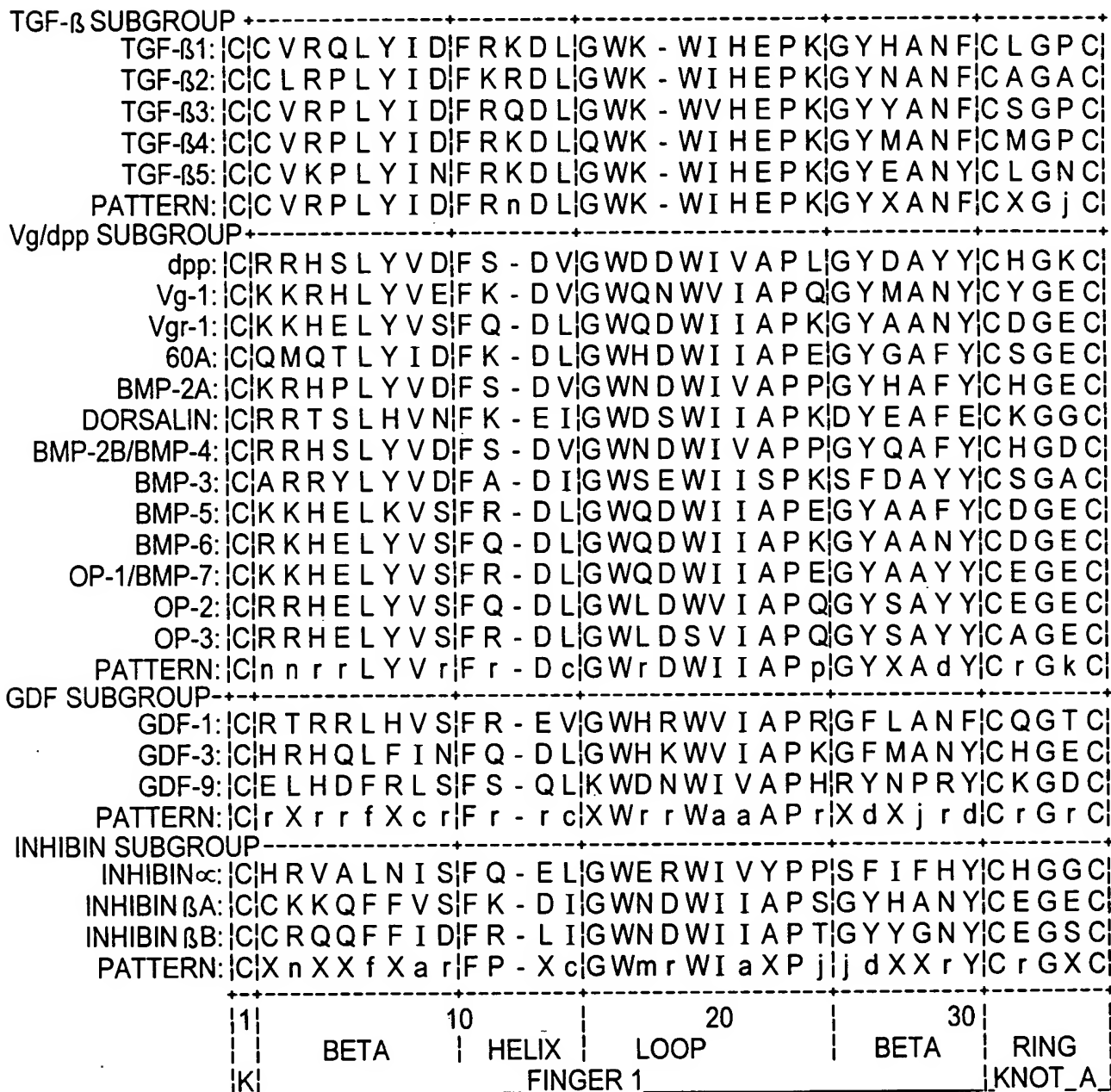


FIG. 5A

TGF- β SUBGROUP									
TGF- β 1:	PY I WS	- - - - -	LDT	QYSKVL	LALYNQHN	P - -	GASAAP	PCIC	
TGF- β 2:	PY L WS	- - - - -	SDT	QHSRVL	SLYNTIN	P - -	EASASP	PCIC	
TGF- β 3:	PY L RS	- - - - -	ADT	THSTVL	GLYNTLN	P - -	EASASP	PCIC	
TGF- β 4:	PY I WS	- - - - -	ADT	QYTKVL	LALYNQHN	P - -	GASAAP	PCIC	
TGF- β 5:	PY I WS	- - - - -	MDT	QYSKVL	SLYNQNN	P - -	GASISP	PCIC	
PATTERN:	PY c WS	- - - - -	XDT	Qe S n VL	j LYNr XN	P - -	XASA j	PCIC	
Vg/dpp SUBGROUP									
dpp:	P F P L A D H F	- - - -	NST	NH A V V Q T L	V N N M N	P - -	G K V P K A	C I C	
Vg-1:	P Y P L T E I L	- - - -	NGS	NH A I L Q T L	V H S I E	P - -	E D I P L P	C I C	
Vgr-1:	S F P L N A H M	- - - -	NAT	NH A I V Q T L	V H L M N	P - -	E Y V P K P	C I C	
60A:	N F P L N A H M	- - - -	NAT	NH A I V Q T L	V H L L E	P - -	K K V P K P	C I C	
BMP-2A:	P F P L A D H L	- - - -	NST	NH A I V Q T L	V N S V N	P - -	S K I P K A	C I C	
DORSALIN:	F F P L T D N V	- - - -	TPT	KH A I V Q T L	V H L Q N	P - -	K K A S K A	C I C	
BMP-2B/BMP-4:	P F P L A D H L	- - - -	NST	NH A I V Q T L	V N S V N	P - -	S S I P K A	C I C	
BMP-3:	Q F P M P K S L	- - - -	KPS	NH A T I Q S L	V R A V G	V V -	P G I P E P	C I C	
BMP-5:	S F P L N A H M	- - - -	NAT	NH A I V Q T L	V H L M F	P - -	D H V P K P	C I C	
BMP-6:	S F P L N A H M	- - - -	NAT	NH A I V Q T L	V H L M N	P - -	E Y V P K P	C I C	
OP-1/BMP-7:	A F P L N S Y M	- - - -	NAT	NH A I V Q T L	V H F I N	P - -	E T V P K P	C I C	
OP-2:	S F P L D S C M	- - - -	NAT	NH A I L Q S L	V H L M K	P - -	N A V P K A	C I C	
OP-3:	I Y P L N S C M	- - - -	NST	NH A T M Q A L	V H L M K	P - -	D I I P K V	C I C	
PATTERN:	X F P L X X X b	- - - -	N j T	NH A I a Q T L	V r X c r	z z -	r X a P K j	C I C	
GDF SUBGROUP									
GDF-1:	A L P E T L R G P G G P P A L		NH A V L R A L	M H A A A	P T -	P G A G S P	C I C		
GDF-3:	P F S M T T Y L	- - - -	NSS	NY A F M Q A L	M H M A D	I - -	P K V P K A	V I C	
GDF-9:	P R A V R H R Y	- - - -	GSP	V H T M V Q N I	I Y E K L	D - -	P S V P R P	S I C	
PATTERN:	j X j X r X X X z z z z X j X		X e j f c p X c c e X X X		z z -	P X X j r j	X I C		
INHIBIN SUBGROUP									
INHIBIN α :	G L H I P P N L S L	- -	PVP	G A P P T P A Q P Y S L	-	- - -	L P G A Q P	C I C	
INHIBIN β A:	P S H I A G T S G S	- -	S L S	F H S T V I N H Y R M R G H			S P F A N L K S	C I C	
INHIBIN β B:	P A Y L A G V P G S	- -	A S S	F H T A V V N Q Y R M R G L N	-		P G T V N S	C I C	
PATTERN:	j X e c j j X X j X	- -	j X j	X X j j X X X r X X X X z		z z z X j X X r j		C I C	

FIG. 5B

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TGF- β SUBGROUP															
TGF- β 1:	V - - PQA	LEPLPIVY	YVG - - RKP												
TGF- β 2:	V - - SQD	LEPLTILY	YIG - - KTP												
TGF- β 3:	V - - PQD	LEPLTILY	YVG - - RTP												
TGF- β 4:	V - - PQT	LDPLPIIY	YVG - - RNV												
TGF- β 5:	V - - PDV	LEPLPIIY	YVG - - RTA												
PATTERN:	V - - PQX	LEPL j I c Y	YVG - - R r j												
Vg/dpp SUBGROUP															
dpp:	V - - PTQ	LD SVAMLY	LNDQ - STV												
Vg-1:	V - - PTK	MSPISMLY	FYDNN - DNV												
Vgr-1:	A - - PTK	LNAISVLY	FDDN - SNV												
60A:	A - - PTR	L GALPVL	YHLND - ENV												
BMP-2A:	V - - PTE	LSAISMLY	LDEN - EKV												
DORSALIN:	V - - PTK	LDAISILY	KDDAGVPT												
BMP-2B/BMP-4:	V - - PTE	LSAISMLY	LDEY - DKV												
BMP-3:	V - - PEK	MSSLSILF	FDEN - KNV												
BMP-5:	A - - PTK	LNAISVLY	FDDS - SNV												
BMP-6:	A - - PTK	LNAISVLY	FDDN - SNV												
OP-1/BMP-7:	A - - PTQ	LNAISVLY	FDDS - SNV												
OP-2:	A - - PTK	LSATSVLY	YDSS - NNV												
OP-3:	V - - PTE	LSAISLLY	YDRN - NNV												
PATTERN:	X - - PT	pL r A a S c L Y	f D m r z r r V a L n r Y p l M X V p j												
GDF SUBGROUP															
GDF-1:	V - - PER	LSPISVLF	FDNS - DNV												
GDF-3:	V - - PTK	LSPISMLY	QDSD - KNV												
GDF-9:	V - - PGK	YSPLSVLT	IEPD - GSI												
PATTERN:	V - - PX	n f S P c S c L X	X k X r - X r a X f n r Y E D M a X r p C j C X												
INHIBIN SUBGROUP															
INHIBIN α :	A A L	P G T M R P L H V R T	T S D G G Y S F K Y E T V P N L L T Q H												
INHIBIN β A:	V - - PTK	L R P M S M L Y	Y D D G - Q N I I K K D I Q N M I V E E												
INHIBIN β B:	I - - PTK	L S T M S M L Y	F D D E - Y N I V K R D V P N M I V E E												
PATTERN:	X z z P j r b r j b r c X X	X r D X z X r f	X X p r a X N b c X o r C h C X												
<table border="1"> <tr> <td>80</td><td>90</td><td>100</td><td>110</td></tr> <tr> <td>BETA</td><td>LOOP</td><td>BETA</td><td>RING</td></tr> <tr> <td colspan="3">FINGER_2</td><td>KNOT2C</td></tr> </table>				80	90	100	110	BETA	LOOP	BETA	RING	FINGER_2			KNOT2C
80	90	100	110												
BETA	LOOP	BETA	RING												
FINGER_2			KNOT2C												

FIG. 5C

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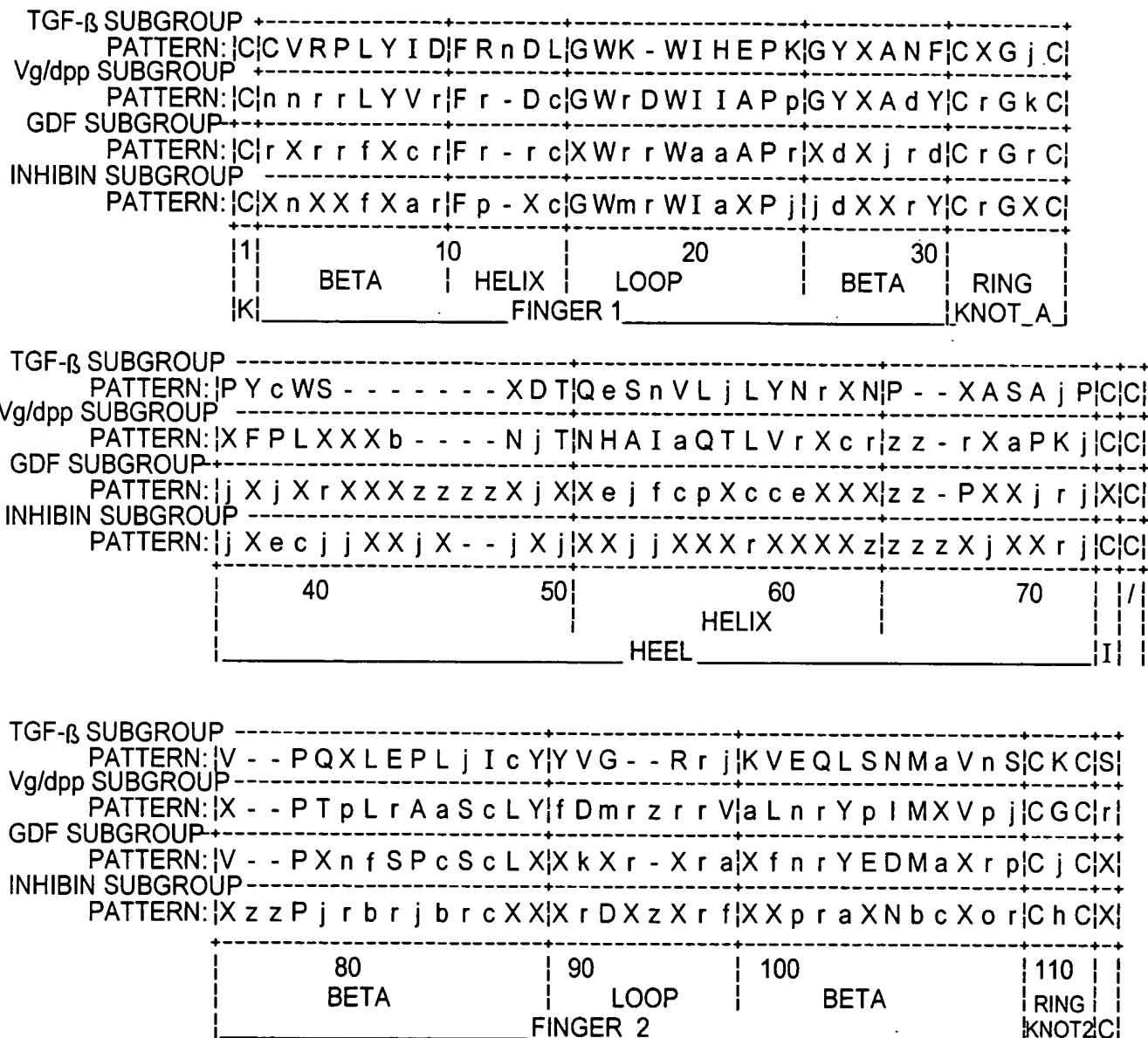


FIG. 6

pH2487

[N-TERMINAL LEADER] COLLAGEN BINDING SITE OP-1 7-CYSTEINE DOMAIN

M T M I T N S L A S I W R E P S F M A L S S D Q R Q A I C K K H E L Y V S F R D L

ATGACCATGATTACGAATCCCTGGCCAGCTGGAGAGAGCCCAAGCTTCATGGCCTTAAGCAGCAGCAGCAGCAGGCTGTAAAGAACGACGAGCTGTATGTCAGCTTCCGAGACCTG

EcoRI MscI HindIII AflII StuI

G W Q D W I I A P E G Y A A Y Y C E G E C A F P L N S Y M N A T N H A I V Q T L

GGCTGGCAGGACTGGATCATCGCGCCTGAAGGCTACGCCGCTACTACTGTGAGGGGGAGTGTGCCCTCTGAACTCCTACATGAACGCCACCAACCCGCGCATCGTGCAGACGCTG

AlwNI

V H F I N P E T V P K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y E D

GTCCACTTCATCAACCCGGAACGGTGGCCCAAGCCCTGCTGTGCGCCCAACGCGAGCTCAGCGCTATCTCCGTCCTCTACTTCGATGACAGCTCCAACGCTCCTGAAGAAATACGAAGAC

AvaII BspI Eco47III DrdI

M V V E A C G C R

ATGGTGGTCGAAGCTTGTGGCTGCAGATAGCTCCTCCGAGAAATC

HindIII PstI EcoRI

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FIG. 7A

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pH2440 His-6 ATTACHED AT 35 RESIDUES UPSTREAM OF FIRST CYSTEINE; POOR ACTIVITY!?

10 20 30
 -CCATGGCTGACAACCATCATCACCATATG
 ... M A D N H H H H H M
 NcoI:1 NdeI:2

40 50 60 70 80 90 100 110 120 130 140
 GGGAGCAACAGCGCAGCCAGAACCGCTCCAAAGACGCGCCCAAGAACCAAGAGCCCTGGCGGATGGCCCAACGTGGCAGAGAGAACAGCAGCAGCAGCAGGCGCC
 G S K Q R S Q N R S K T P K N Q E A L R M A N V A E N S S S D Q R Q A
 BsaHI:2 BglI:7 OP-1-exon5----- StuI
 MscIdcm:b

FIG. 7B

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pH2521 FB LEADER, AND 15 RESIDUES UPSTREAM FROM FIRST CYSTEINE

10 20 30 40 50 60 70 80 90 100 110 120
ATGATCGAATTCATGGCTGACACAAATTCACAAAGGAACAGACGCGTTCTACGAGATCTTGCACCTGCCGAACTGAACGAAAGAGCAGCGCTTAACGGCTTCATCCAAAGCCTGAAA
M I E F M A D N K F N K E Q Q N A F Y E I L H L P N L N E E Q R N G F I Q S L K

EcoRI:1

MluI:1

BglII:1

XmnI:b

130 140 150 160 170 180 190
GAAGAGCCGCTCAGCTCGGAATCTGCTAGCGGATGCCAAGAACTGAACGATGCCGAGGCACCGAAATCGGCC
E E P S Q S A N L L A D A K K L N D A Q A P K S A

NheI:1

FspI:b

300 310 320 330 340
ATGGCAACGTGGCAGAGAACAGCAGCAGCGACCGACCCAGAGGCAGGCCT
M A N V A E N S S S D Q R Q A

BglI:7

op-1-exon5-----

StuI

NcoI:1

XcmI:8

MscIdcm:b

FIG. 7C

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pH2525 FB- AND His6-LEADER, RETAINING 35 RESIDUES UPSTREAM FROM FIRST CYSTEINE; GOOD REFOLDING

ATGATCGAAATTCATGGCTGACAAACAAATTCACAAAGGAACAGCAGAACGCGTTCTACGAGATCTTGCACTGCCGAACCTGAACGAAGAGCAGCGTAACGGCTTCATCCAAAGCCTGAAA
M I E F M A D N K F N K E Q Q N A F Y E I L H L P N L N E E Q R N G F I Q S L K
EcoRI:1 MluI:1 XmnI:b BglII:1

GAAGAGCCGTCTCAGTGTGCGAATCTGCTAGCGGATGCCAAGAACTGAACGATGCCAGGACCCGAAATCGGCCATGGCTGACAACCATCACCATCATCACCATATG
E E P S Q S A N L L A D A K K L N D A Q A P K S A M A D N H H H H H H M
NheI:1 FspI:b NcoI:1 NdeI:2

GGGAGCAAACAGCGCAGCCAGAACCGCTCCAAGACGCCCCAAGAACCCAGGAAGCCCTGCGGATGGCCACGTCGCGAGAGAACAGCAGCAGCGACCGAGAGGCAGGCCT
G S K Q R S Q N R S K T P K N Q E A L R M A N V A E N S S S D Q R Q A
MscI StuI

FIG. 7D

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pH2527 FB-His-6-TRUNCATED OP-1 WITH ACID CLEAVAGE SITE

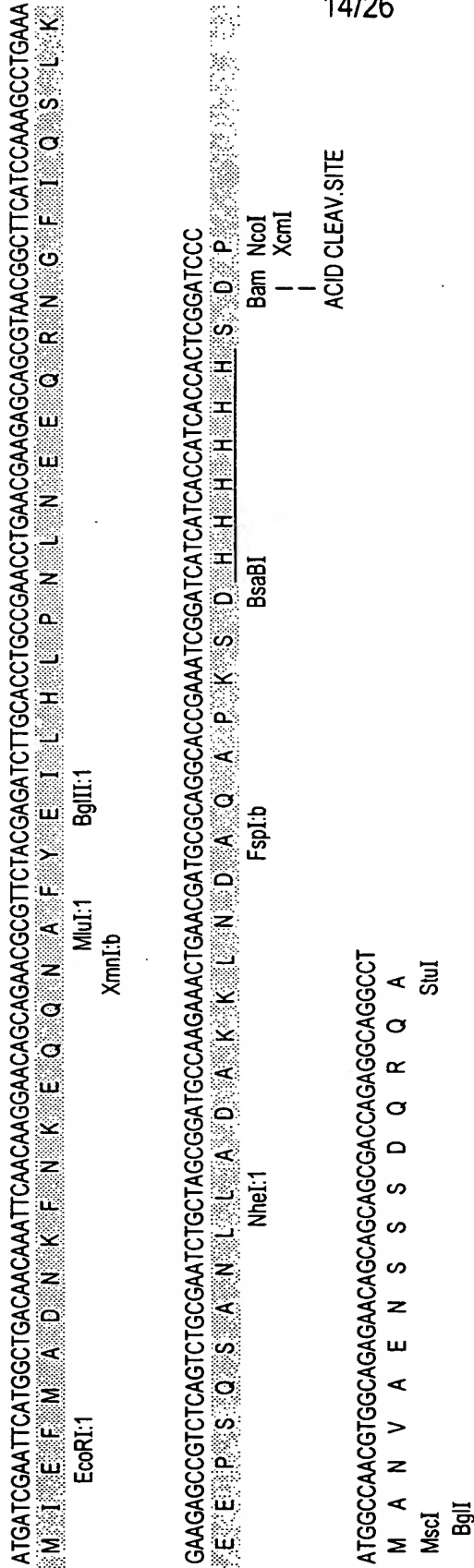


FIG. 7E

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H2528
FB-His6-CHIMP-3

```
10      20      30      40      50      60      70      80      90     100     110     120
-CCATGATCGAATTCATGGCTGACAAACAAATTCAACAAGGAACAGCAGACGCGTTCTACGAGATCTTGACACCTGCCGAACTGAACGAGCAGCGTAACGGCTTCATCCAAAGCCTG
... M I E F M A D N K F N K E Q Q N A F Y E I L H L P N L N E E Q R N G F I Q S L

130     140     150     160     170     180     190     200     210     220     230     240
AAGAAGAGCCGCTCAGTCTGCGAATCTGCTAGCGGATGCCAAGAACTGAACGATGCGCAGGCACCGAAATCGGATCATCATCACCATCACCACCTCGGATGCCGTTGGCCCGGG
K E E P S Q S A N L L A D A K K L N D A Q A P K S D H H H H H S D P M A L A G

250     260     270     280     290     300     310     320     330     340     350     360
ACGCGTACAGCGCAGGGCAGCGGAGGTGCCGGCAGAGGTTCATGGTCGACGTGGTAGATCTCGCTGCAGCCGCAAGCCGTTGCACGTGGACTTCAAGGAGCTCGGCTGGGACGACTGG
T R T A Q G S G G A G R G H G R G R G R S R C S R K P L H V D F K E L G W D D W

370     380     390     400     410     420     430     440     450     460     470     480
ATCATCGCGCGCTGGACTACGAGGCGTACCACCTGCGAGGGGCTTTGCGACTTCCCTTTCGCGTTGCGACCTCGAGCCCAACCAACCATGCCATCATTCAGACGCTGCTCAACTCCATGGCA
I I A P L D Y E A Y H C E G L C D F P L R S H L E P T N H A I I Q T L L N S M A

490     500     510     520     530     540     550     560     570     580     590     600
CCAGACGCGCGCGGCTCCTGCTGTGTGCCAGCGCGGCTCAGCCCCCATCAGCATCCTTACATCGACGCGCCCAACAACGTTGTCTACAAGCAATACGAGGACATGGTGGTGGAGGCC
P D A A P A S C C V P A R L S P I S I L Y I D A A N N V V Y K Q Y E D M V V E A

610     620     630     640     650
TGGCGCTGTAGGTAAGCTTGTGGCTGCAGATAGCTCCTCCGAGAATTC
C G C R *
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FIG. 7F

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pH2469 TRUNCATED, GOOD ROS ACTIVITY; 14 ORIGINAL RESIDUES UPSTREAM OF FIRST CYSTEINE

	10	20	30	40	
-CCATGGCCCAACGTGGCAGAGAACACAGCAGCAGCGACCGACCGAGAGGGCAGGCC					
... M A N V A E N S S S D Q R Q A					
NcoI BglI:7			OP-1-exon5-----		StuI
MscIdcm:b					

FIG. 7G

pH2510 COLLAGEN SITE INSERTED 7 RESIDUES UPSTREAM OF CYSTEINE; GOOD EXPRESSION, REFOLDING

10 20 30 40 50 60 70 80 90 100 110
ATGTCACGGGGAGCAACAGCGCAGCGCAACCGCTCCAAGACGCCCAAGAACCAAGAGCCCTGCGGATGGCCAGCTTCAATGGCCCTTAAGCAGC
M S T G S K Q R S Q N R S K T P K N Q E A L R M A S W R E P S F M A L S S
BsaHI:2 BpmI+ HindIII:1 AflII:1
120 130 MscIdcm:b BfrI:1
AGCGACCAGAGCGCGCC PvuII
S D Q R Q A StuI

FIG. 7H

pH2523 COLLAGEN PEPTIDE, AND SPACER ADDED AT 13 RESIDUES UPSTREAM FROM 1ST CYSTEINE

10 20 30 40 50 60 70 80 90 100 110
ATGTCCACGGGGAGCAACAGCGCAGCGCAACCGCTCCAAGACGCCCAAGAACCAAGAGCCCTGCGGATGGCCAGCTTCAATGGCCCTTAAGCAGCGCGAC
M S T G S K Q R S Q N R S K T P K N Q E A L R M A S W R E P S F M A L S S D
BsaHI:2 BpmI+ HindIII:1 AflII:1
120 130 140 150 160 BfrI:1
CAGAGGAGCGCCCAACGTGGCAGAGAACAGCAGCAGCGCGCAGCGCGAGCGCGCC
Q R Q A N V A E N S S S D Q R Q A DUPLICATION
BglI OP-1-exon5----- StuI

FIG. 7I

pH2524 Hexa-His, COLLAGEN PEPTIDE, SPACER ADDED AT 13 RESIDUES UPSTREAM FROM 1ST CYSTEINE

10 20 30
-CCATGGCTGACAAACCATCACCATCATCACCATATG
... M A D N H H H H M
NcoI:1 NcoI:2
40 50 60 70 80 90 100 110 120 130 140
GGGAGCAACAGCGCAGCGCAACCGCTCCAAGACGCCCAAGAACCAAGAGCCCTGCGGATGGCCAGCTTCAATGGCCCTTAAGCAGCGCGCGAC
G S K Q R S Q N R S K T P K N Q E A L R M A S W R E P S F M A L S S D Q
BsaHI:2 BpmI+ HindIII:1 AflII:1
150 160 170 MscIdcm:b BfrI:1
AGGCGGCCCAACGTGGCAGAGAACAGCAGCAGCGCGCAGCGCGAGCGCGCC
R Q A N V A E N S S S D Q R Q A DUPLICATION
BglI OP-1-exon5----- StuI

FIG. 7J

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7-CYSTEINE DOMAIN OF OP-1

FINGER-1

TGTAAGAAGCACGAGCTGTATGTCAGACTTCCGAGACCTGGGCTGGCAGGACTGGATCATCGCGCTGAAGGCTACGCCGCCCTACTACTGTGAGGGG
C K K H E L Y V S F R D L G W Q D W I I A P E G Y A A Y Y C E G

HEEL

GAGTGTGCCCTTCCCTCTGAACCTCTACATGAACGCCCAACCAACCGCCATCGTGCAGACGCTGGTCCACTTCATCAACCCGGAAACGGTGCCCAAGCCCTGC
E C A F P L N S Y M N A T N H A I V Q T L V H F I N P E T V P K P C

FINGER-2

TGTGCGCCACGACGCTCAATGCCATCTCCGTCCTCTACTTCGATGACAGCTCCAACGTCATCCTGAAGAAATACAGAAACATGGTGGTCCGGGSCCTGTGGCTGCCAC
C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C H

FIG. 8

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OP-1 CHIMERICS WITH CDMP-2 OR WITH BMP-2

PARENTAL MOLECULES: REFOLDING ACTIVITY (CELL BASED)

	FINGER1	HEEL	FINGER2			
OP-1				h	(-)	+++ (*)
BMP-2				r	+++	+++
CDMP-2				r	++++	+/-

REPLACING FINGER-1 OR HEEL:

H2383				r	+/-	N/A
H2362				r	+	N/A
H2360				r	+	N/A
H2331				r	+	N/A

REPLACING FINGER-2 OR HEEL:

H2389				r	+++	+++
H2471				r	+++	+++
H2388				r	+++	+/-
H2410				r	+++	+++
H2429				r	+/-	N/A

CHANGING PATCHES OF RESIDUES:

H2381				r	+++	N/A
H2390				r	+	N/A
H2396				r	+	N/A
H2421				r	+/-	N/A

PAIRED CHANGES IN FINGER-2:

H2418				r	+++	++
H2420				r	++++	+/-

FIG. 9A

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OP-1 MUTANTS WITH C-TERMINAL ARGININE INSTEAD OF HISTIDINE:

H2247		+	+++
H2233		+	+++

BALANCING OF CHARGED RESIDUES IN FINGER-2 OF OP-1 MUTANTS:

Strain	Genotype	Phenotype	Notes
H2406	1,4,6,7 vktp r	+/-	N/A
H2443	1,4,6,7 25,26 vktp ed r	+++	++
H2447	1,4,6 25,26,30 ves ede r	+++	++
H2433	4 k r	+/-	N/A
H2456	4,6 25,26,30 es ede r	+++	+++

FIG. 9B

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CORRELATION OF REFOLDING EFFICIENCY AND CHARGED AMINO ACIDS
IN THE TGF- β (SEVEN CYSTEINE) DOMAIN

PROTEIN	FINGER-1	CXGXC	HEEL	FINGER-2	CXCX C-TERM	TOTAL OF CHARGED RESIDUES (+), (-) = TOTAL	NEGATIVE CHARGES, FINGER-2	NET CHARGES, FINGER-2	REFOLDING EFFICIENCY
OP-1	3+, 4-	2-	1+, 1-	4+, 2-	0	8+, 9- = 17	2-	2+	+/-
H2247	3+, 4-	2-	1+, 1-	4+, 2-	1+	9+, 9- = 18	2-	2+	+
H2447	3+, 4-	2-	1+, 1-	2+, 6-	1+	7+, 12- = 19	6-	4-	+++
BMP-3	4+, 4-	0	3+, 1-	3+, 4-	1+	11+, 9- = 20	4-	1-	+++
BMP-2	2+, 3-	1-	2+, 1-	2+, 6-	1+	7+, 11- = 18	6-	4-	+++
GDF-5	3+, 5-	1-	1+, 4-	2+, 4-	1+	6+, 14- = 20	4-	2-	+++
CDMP-2	3+, 5-	1-	1+, 3-	2+, 4-	1+	6+, 13- = 19	4-	2-	+++
GDNF	2+, 4-	0	6+, 4-	5+, 5-	0	13+, 13- = 26	5-	0	+++
TGF- β 1	5+, 3-	0	1+, 1-	5+, 2-	1+	11+, 6- = 17	2-	3+	+/-
TGF- β 2	5+, 3-	0	1+, 2-	4+, 3-	1+	10+, 8- = 18	3-	1+	+/-

FIG. 10

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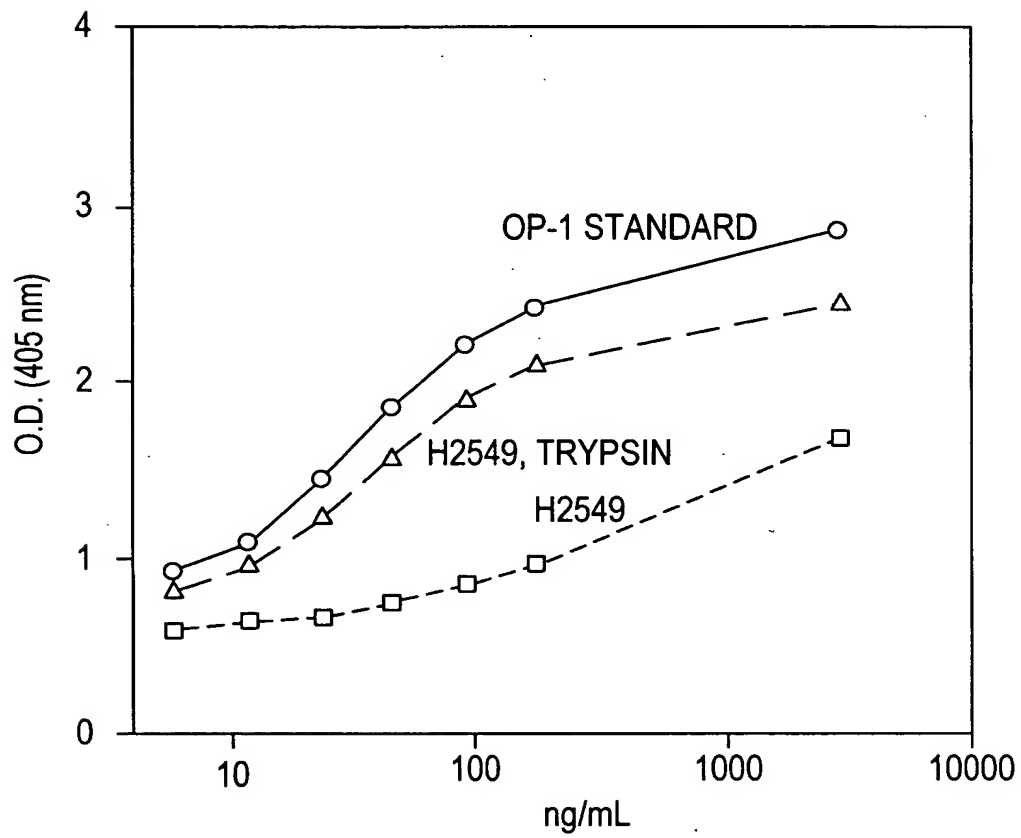
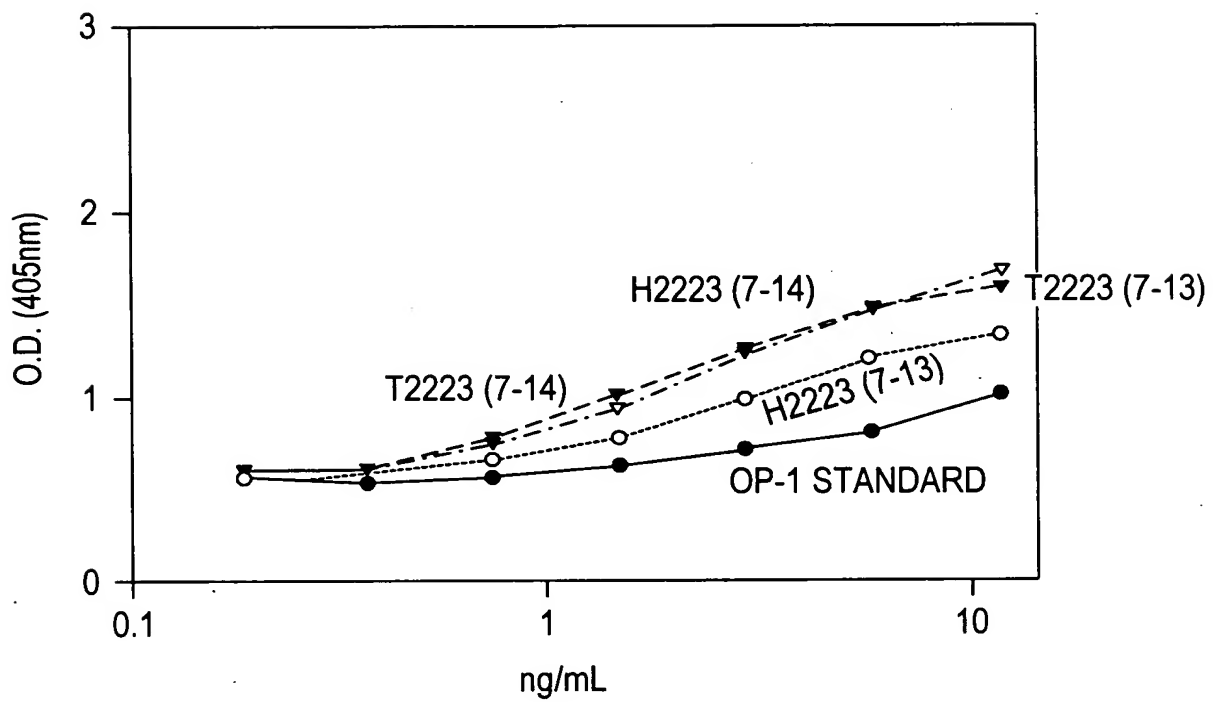


FIG. 11

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**FIG. 12**

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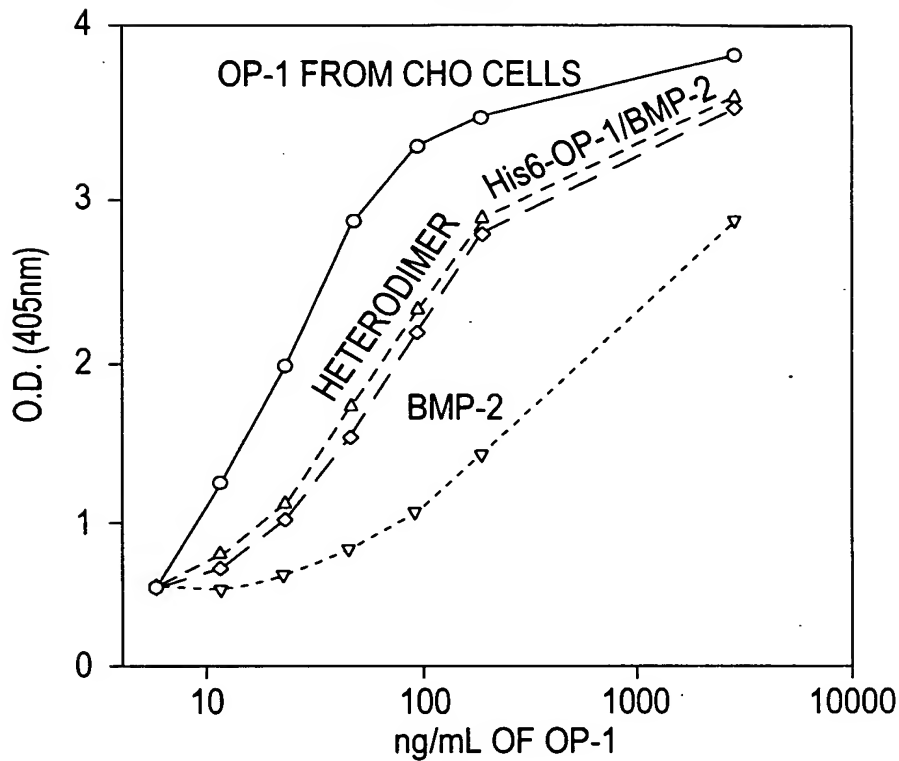


FIG. 13A

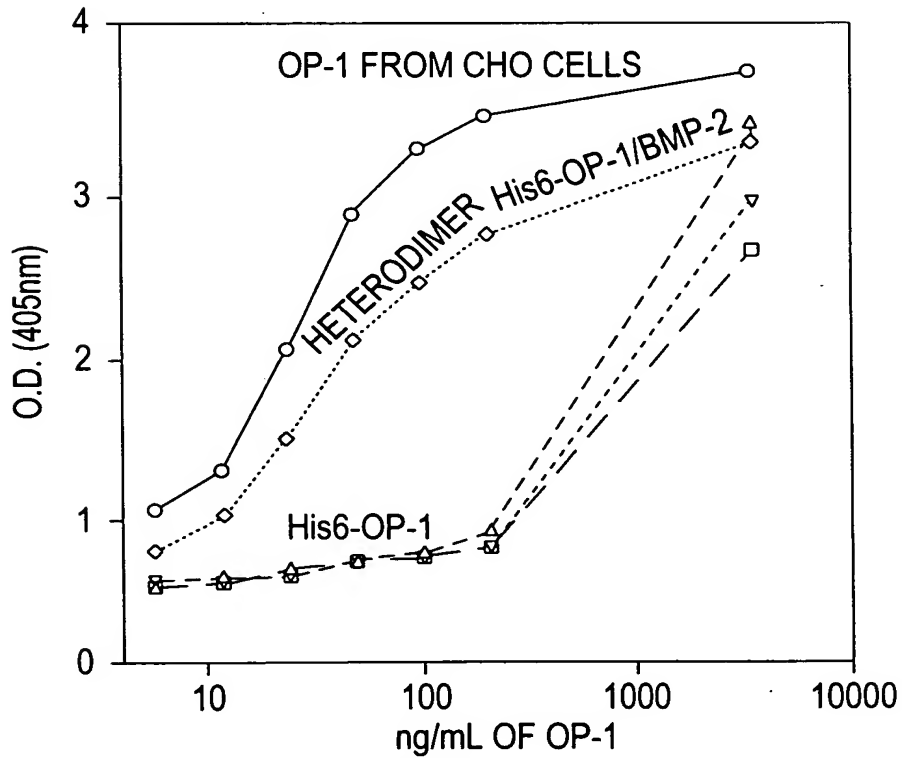


FIG. 13B

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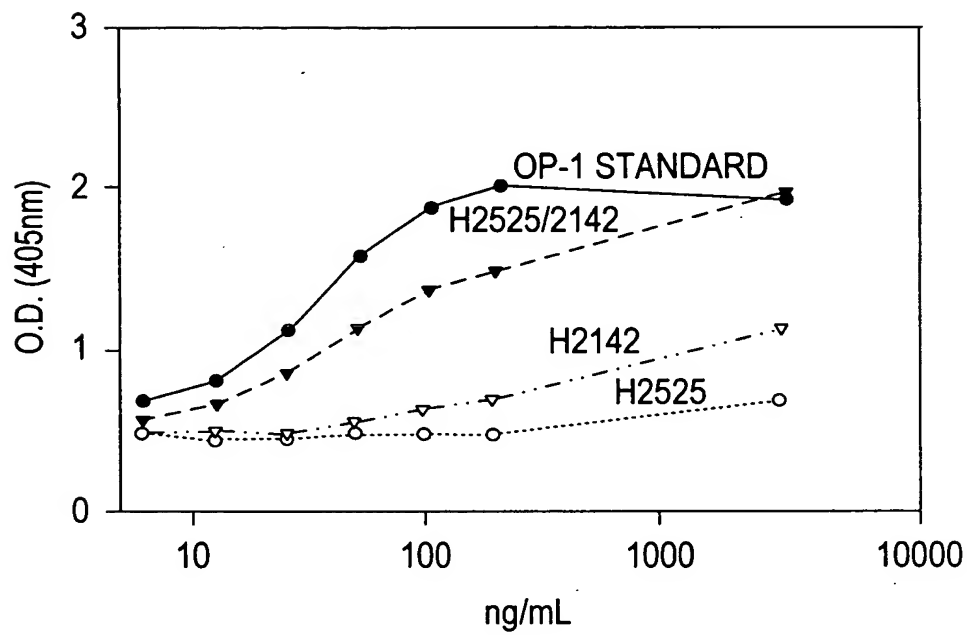


FIG. 14

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CHANGES

FOLDING

ROS

FIG. 15

OP-1	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C H	-	5	[-]
2421	P T C C V P T R L S P I S I L F I D A S N N V V L K K Y R N M V V R A C G C R	(+)		
2406	N S C C V P T K L T P I S I L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)		[8]
2410	N S C C V P T E L S A I S M L Y L D E N E K V V L K N Y Q D M V V E G C G C R	3+	4	[18]
2247	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)	3	[1]
2234	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y E D M V V R A C G C R	(+)		[3]
2233	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	1+	3	[4]
2418	N S C C V P T K L T P I S V L Y F D D S S N V I L K K Y E D M V V E S C G C R	3+	2	[10]
2443	N S C C V P T K L T P I S V L Y F D D S S N V I L K K Y E D M V V R S C G C R	3+		[9]
2447	N S C C V P T E L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+	2	[9]
2457	N S C C V P T E L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	2+	3	[8]
2456	K P C C A P T E L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+	3	[6]
2460	K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+		[5]
2457	N S C C V P T E L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	2+	3	[8]
2449	K P C C A P T E L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	2+	3	[2]
2467	K P C C A P T E L S A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)		[3]
2464	K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)		[2]

FINGER-2 SEQUENCES OF OP-1 MUTANTS AND THEIR FOLDING EFFICIENCIES AND BIOLOGICAL ACTIVITIES IN THE ROS CELL BASED ALKALINE PHOSPHATASE ASSAY.

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